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Research Article

Nutri-Priming as an Efficient Means to Improve Germination and Growth of Mung bean (*Vigna radiata* L.) Grown Under NaCl Stress

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Abstract

Salinity is a major environmental issue that reduced the crop growth and productivity. The micronutrients like Zn have the potential to decrease the toxic effects of salinity. The recent study was conducted to examine to effect of $ZnSO_4$ on germination percentage, physiological, biochemical attributes and antioxidant enzymes (CAT, POD, SOD) activities of *Vigna radiata* L. The seeds were primed with two (100ppm and 200ppm) concentrations of $ZnSO_4$ and then grown under various levels of NaCl (0-50-75-100 mM) stress. The results indicated that various physiological, morphological and biochemical parameters negatively influenced by salt stress. However, the nutripriming with $ZnSO_4$ enhanced the germination percentage and growth of *Vigna radiata* under NaCl stress. The comparison of both concentrations showed that 100ppm improved the morphological parameters (root, shoot length, fresh and dry weight of root, shoot) and Biochemical parameters (proline and total soluble protein), While the nutripriming with 200ppm improved the chlorophyll (a & b), phenolics and flavonoids contents more than control. The Zn application improves the Antioxidant enzymes (CAT, POD, SOD) but at higher doses cause reduction. This is environment friendly technique and can be used in future.

Keywords; Flavonoids; Protein; Proline; Seed priming; Salinity; $ZnSO_4$

Introduction

Mung bean (*Vigna radiata* L.) is one of the most important food crops in Pakistan. It is a legume crop with higher contents of proteins and short summer season crop. The mung bean can be used as an export potential crop [1]. Mung bean is the member of family *Fabaceae* and genus *Vigna*. Mung bean in Pakistan is being cultivated on 146,000 hectares with annual 98,000 tons of production, marginal yield per unit area [2].

The salinity reduced the growth rate and yield of agricultural crops more than other environmental factors [3]. The abiotic stress negatively influenced the development and yield of many crops [4]. The higher levels of salt stress caused adverse effects on growth and yield of crops [5]. More than 800 million/hectors land are badly influenced by salt stress through the world [6].

However, the micronutrients have the potential to reduce the salinity. The most well-known strategies for micronutrient application are foliar application, seed treatment and soil application. The nutripriming is a basic priming strategy in which seeds are absorbed soaked in aerated solution of nutrients [7]. Since micronutrients are required in smaller amounts, priming in solutions with higher concentrations may cause seed damage and poor germination. Therefore, concentration of nutrient solution is to be optimized before field testing [8].

Zinc in the earth crust naturally occurs at 70 mg/kg, which ranges between 10 to 300 mg/kg. It is usually estimated about 30% Zinc is produced from recycled products. The industrial movement has brought about anthropogenic contribution of zinc to the earth [9]. Zinc is absorbed by the roots as divalent cation (zn^{2+}). Zn activates many enzymes like alcohol dehydrogenase (ADH) and carbonic anhydrase (CA). While deficiency of Zn in soil cause changes in metabolism of the auxin hormone. The Zn availability in soil depends on soil pH, presence of micro and macronutrients and soil microorganisms. Their intersection among themselves makes the Zn available and non-available. While increase in soil pH causes the less solubility in soil and its uptake also deceased [10]. Zinc plays essential role in chlorophyll synthesis [11]. The objectives of the recent study are to standardize the methodology of nutripriming of *Vigna radiata* L. with $ZnSO_4$, to examine the role of micronutrients on germination, growth, and biochemical aspects grown under salt stress.

Materials and Methods

Collection of seeds

The seeds of *Mung bean* (*Vigna radiata* L. cv. Chakwal) were collected from NARC (National Agriculture Research Center Islamabad).

Experimental design

The seeds were surface sterilized for 30 seconds with 70% ethanol and then washed with distilled water. The seeds were primed for 1-hour with 100ppm and 200ppm $ZnSO_4$ and then seeds were dried. Later the seeds were sown under NaCl (0-50-75-100mM NaCl) stress. The experiment was placed with 3 replicates and total 36 pots were used. The germination percentage were observed after 24 hours up to 7 days to count constant percentage of germination. The plants of *Vigna radiata* L. were harvested and length of shoot, root was measured with simple ruler, while the fresh and dry weight of root, shoot was measured with electronic measuring balance.

Antioxidant enzymes

The method of Beauchamp and Fridovich [12] was followed to determine the Superoxide dismutase (SOD) activity, protocol of [13] for Peroxidase (POD) activity and method of [14] was used for estimation of Catalase activity.

Biochemical analysis

The protocol of [15] and the following equation were used to calculate the chlorophyll a and chlorophyll b. The contents of proline in leaves of *Vigna radiata* L. were determined by the method of [16] and phenolic contents were estimated using the protocol of [17]. The flavonoids were estimated by following the method of [18] by using $AlCl_3$ protocol. The total soluble protein was determined by using the protocol of [19] and final calculation was done by using following equation;

Protein (mg / g) = OD × K value × Dilution Factor/ sample weight

K value = 19.6

Results and Discussion

The results mentioned in (Table 1) indicated that germination percentage strongly reduced under salt stress. The germination percentage significantly ($P<0.05$) reduced under NaCl stress. Our findings are in consistence with the [20, 21]. The reduction in germination is related to salinity disturbances with the metabolic process causing increase in phenolic compounds. The reduction in germination is also due to reduced water movement into seed during imbibition's [20]. Salt stress influenced the seed germination through osmotic effects [22]. However, nutripriming with $ZnSO_4$ enhanced the seed gemination under NaCl stress. The nutripriming with $ZnSO_4$ increased the germination percentage more than control plants under NaCl stress, while comparison of 100ppm and 200ppm showed that higher levels of Zn also inhibit the germination percentage. The significantly ($P<0.005$) highest germination percentage were observed at 100ppm under 50m MNaCl. The higher emergence was results of reduced.

Physiological non-uniformity in seeds due to priming. Our results are in consistence with the findings of [23, 24], they reported that priming of seeds enhanced the germination rate, faster seedlings emergence and early germination. The nutripriming with Zinc enhanced the germination and seedling growth in barley reported by [25], while the higher concentrations of Zn reduced the germination percentage as reported by [4, 26]. The reduction in germination percentage under higher doses of Zinc were also reported in *Vigna mungo* (variety T-9) by [27] and on *Sesuvium portulacastrum* by [28].

Table 1. Effect of nutripriming with $ZnSO_4$ on germination percentage of *Vigna radiata* L. under NaCl stress (n=3, mean ±value SE)

Treatments	0 mM	50 mM	75 mM	100 mM
Control	a85.71±8.25	b76.18±4.76	b71.42± 0.00	b65.66 ±4.76
$ZnSO_4$ 100ppm	a95.23± 4.76	a100± 0.00	a95.23± 4.76	a85.71 ±0.00
$ZnSO_4$ 200ppm	a95.23±4.76	ab90.47± 4.76	ab85.71 ±8.25	ab80.94± 4.76

The results mentioned in fig. 1A showed that salt stress negatively influenced the shoot length. The shoot length of *Vigna radiata* L. significantly ($P<0.05$) reduced under NaCl stress. Our findings are in consistence with [29, 30]. The reduced growth of shoot may be because of injurious effects of sodium chloride and unbalanced uptake of nutrients by plants. The higher levels of salt stress Inhibit shoot and root elongation by disturbance in water uptake by plant. However, the nutripriming with $ZnSO_4$ significantly ($P<0.05$) increased the shoot length under NaCl stress. While the comparison of 100ppm and 200ppm showed that Zn at lower levels improves the plant growth but at higher concentration proves toxic and disturbs the plants growth. The maximum significant ($P<0.05$) values of shoot length (35.50±0.12) were observed at 100ppm under 50mM NaCl. Zinc sulphate generally inhibits physiological processes. As zinc interference with the activities of many enzymes essential for normal development and metabolic processes and due to its direct interaction with proteins pigments, etc. [31]. Zn is an important micronutrient for plant growth, but extent amounts proves toxic and can disturbs the normal biological processes [32]. The lower doses of Zn improved the plant growth but at higher concentrations reduction in plant growth were found [33].

The findings of present study mentioned in fig. 1B indicated that fresh weight of shoot significantly ($P<0.05$) decreased under increased levels of various salt stress. The findings of recent study are in consistence with [34], they found that salinity lowers the fresh weight of pumpkin genotypes. However, the nutripriming with $ZnSO_4$ significantly ($P<0.05$) improved fresh biomass of shoot under NaCl stress. The comparison of 100ppm and 200ppm showed that higher levels of Zn proves toxic, while the highest value (25.37±0.32) of shoot fresh weight was found at 100ppm under 50mM NaCl. Zinc at lower concentration improves the germination, fresh and dry weight over the control and Zn at lower concentrations role as a significant stimulatory, beneficiary and nutritional micronutrient. Zn treatment improves the shoot length and shoot weight of soybean grown under NaCl stress **[9]**. The higher doses of Zn lower the root, shoot fresh and dry weight **[35]**.

The findings of recent study mentioned in fig. 1C showed that salt stress strongly influenced the shoot dry weight. There was a significant ($P<0.05$) reduction in the shoot dry weight under NaCl stress. Our findings are supported by **[30]**. However, the nutripriming with $ZnSO_4$ promoted dry weight of shoot under various levels of NaCl. The comparison of 100ppm and 200ppm showed that nutripriming with $ZnSO_4$ at 100ppm promoted this parameter more than control and priming with 200ppm. The significant ($P<0.05$) highest (8.47 ± 0.06) values of shoot dry weight were found at 100ppm under 50mM NaCl. Which showed that Nutripriming with Zn improves the plant growth at lower levels, but higher levels of Zn caused detrimental effects over plants. Our results are in conformity with **[35, 36]**, they reported that Zinc improves fresh and dry weight and proline contents.

The findings mentioned in fig. 1D showed that root length of *Vigna radiata* L. is negatively influenced by salt stress. The root length significantly ($P<0.05$) reduced under NaCl stress. The decrease in root length under various levels of NaCl were also described by **[29, 30]**. Salt stress inhibit the root growth more than shoot, as roots are directly contacted to soil and absorbs water and supply to rest of plants **[37]**. However, the nutripriming with $ZnSO_4$ significantly ($P<0.05$) improved the root length under NaCl stress. The comparison of 100ppm and 200ppm showed that the nutripriming with 100ppm promoted the root length under NaCl stress more as compared to priming with 200ppm and control plants. The significant ($P<0.05$) maximum values of root length (20.33 ± 1.26) were found at 100ppm under 0mM NaCl stress. Which showed that higher concentrations of Zn caused injurious effects. At lower concentration it improves the plant growth but higher doses reduction in plant growth were found **[33]**.

The results mentioned in fig. 1E showed that there was a significant ($P<0.05$) reduction in fresh weight of root under NaCl stress. The salt stress negatively influenced the rot fresh weight and minimum values were observed at higher level (100mM) of NaCl. Our findings are in conformity with **[30]**. However, the nutripriming with $ZnSO_4$ enhanced the root fresh weight under salt stress. The comparison of both (100ppm & 200ppm) concentration showed that 100ppm improved the root dry weight more than 200ppm. The significant ($P<0.05$) higher fresh weight of root was found (4.09 ± 0.036) at 100ppm under 0mM NaCl. Our findings are in consistence with **[9, 35, 36]**.

The results mentioned in fig. 1F indicated root dry weight is strongly reduced under NaCl stress. The higher levels of salt stress significantly ($P<0.05$) decrease the dry weight of root. Our results are supported with the findings of **[30]**. While the nutripriming with Zn improves the dry weight of root of *Vigna radiata* under salt stress. The comparison of 100ppm and 200ppm sowed that nutripriming with 100ppm $ZnSO_4$ significantly ($P<0.05$) improved dry weight of root more as compared to 200ppm under salt stress. The higher levels of Zn also cause reduction in root length and in return fresh and dry weight of root. **[9, 36]**.

The results mentioned in fig. 2A& B Showed that photosynthetic pigments decreased at higher levels of salt stress. The higher levels of NaCl caused toxic effects on chlorophyll (a & b). The decrease in photosynthetic pigments under NaCl stress may be because of injurious effects of accumulated (Na^+& Cl^-) on biosynthesis of pigments and chloroplast structure. In this regard **[30]** reported that salinity influences the strength of binding forces of pigmentprotein yield complex in the structure of chloroplast. Salinity also caused swelling of membrane in chloroplast and thus effects the chlorophyll and excess ion in leaves induces loss of chlorophyll [38]. However, the nutripriming with $ZnSO_4$ significantly ($P<0.05$) increased chlorophyll (a & b) contents under salt stress. While the comparison of both (100ppm & 200ppm) concentrations showed that 100ppm increased the chlorophyll (a & b) contents more as compared to 200ppm under salt stress. The effects of salt stress were less effective on photosynthetic pigments of plants treated by Zn and Zn improve the chlorophyll a, b of *Vicia faba* (cv. Giza-716) reported by [38]. The positive effects of Zn to improve chlorophylls were also reported by [36, 39]. Zn improves the growth and chlorophyll contents of tomato plants under salinity stress [40, 41].

The salt stress strongly influenced the proline contents of leaves of *Vigna radiata* L. The fig. 2C showed that proline contents are accumulated under salt stress. Our results are supported by the findings of [30], while the [26] suggested that accumulation of proline is used as a sensitive index of susceptibility to water stress. However, in a few plants' species the NaCl stress stimulated the antioxidative enzymes activities, that showed the role of salt stress in formation of ROS [42].The comparison of both (100ppm & 200ppm) concentrations showed that 100ppm significantly ($P<0.05$) increased the proline contents under salt stress. The significantly ($P<0.05$) higher values of proline contents (76.36±1.48) were found at 100ppm under 100mM NaCl. Proline is accumulated under heavy metals stress and considered too involved in stress resistance [43]. The accumulation of proline helps to utilize the nitrogenous compounds and protects plants against heavy metals stress. The accumulated proline also acts as membrane stabilizing agent under stress [44].

The results mentioned in fig. 2D showed that phenolic contents also accumulated under salt stress. The phenolic contents are directly proportional to salt stress, with the increased NaCl levels the phenolic contents also increased. While the nutripriming with Zn improved the phenolic contents under salt stress. The comparison of both (100ppm & 200ppm) was made and findings showed that 200ppm significantly ($P<0.05$) improved the phenolic contents. The significant higher values of phenolic contents (94.37±1.24) were found at 200ppm under 100mM NaCl. Our results are in consistence with [38, 44], who reported the role of Zn to improves the total phenolic contents.

The flavonoid contents are strongly influenced by salt stress. The findings mentioned in fig. 2E showed that flavonoids contents increased under salt stress. The comparison of both (100ppm & 200ppm) was made and findings showed that 200ppm significantly ($P<0.05$) increased the flavonoids contents. The significant ($P<0.05$) higher values of flavonoids contents (74.18±1.67) were found at 200ppm under NaCl stress. Our results are in consistence with [1, 38, 44].

The results mentioned in fig. 2F showed that total Soluble Protein significantly ($P<0.05$) effected by NaCl stress. It was found that protein is accumulated under salt stress and when the NaCl levels increased then total soluble protein also increased. While the nutripriming with $ZnSO_4$ reduced the protein in *Vigna radiata* L. under salt stress. The comparison of both (100ppm & 200ppm) was made and findings showed that 100ppm increased the protein contents. The significant ($P<0.05$) higher values of protein (0.432±0.054) were found at 100ppm under 100mM NaCl stress. Zn has essential role in protein and nucleic acid synthesis and for N and P utilization in formation of seed and development [45]. The excess of Zn decreased the protein because metals bind with the sulfhydryl group of protein causing deleterious effect in the normal protein form [11].

The fig. 3A showed that CAT activity is strongly affected by salinity. It was

observed that CAT Activity was significantly ($P<0.05$) higher in the leaves of plants emerged from the primed seeds grown under salt stress than control plants. While comparison of both (100ppm & 200ppm) concentrations showed that higher doses of Zn also cause reduction in the CAT activity. Our results are in conformity with [46, 47]. The application of Zn improves the CAT activity, but higher doses of Zn also cause reeducation in CAT activity as described by [48] in the wheat.
The fig. 3B showed that POD activity is also strongly affected by salinity. It was observed that POD activity significantly increased under salt stress. While comparison of both (100ppm & 200ppm) concentrations showed that higher doses of Zn cause reduction in the POD activity. Our results in conformity with [46, 49, 50]. Salt stress is the major cause to increase the concentrations of ROS. Therefore, antioxidant enzymes play significant role to remove destructive oxidant species [47].
The fig. 3C showed that SOD is directly proportional to salt stress. As the salt stress increased the SOD activity also increased. The comparison of both (100ppm & 200ppm) concentrations showed that higher doses of Zn cause reduction in the SOD activity. The significant maximum SOD activity was observed at 100ppm under 100mM. Our results in conformity with [50, 51]. Salt stress cause the production of ROS. To reduce the oxidative stress plant, adopt different defensive mechanism like enzymatic and non-enzymatic antioxidant system [52]. The antioxidant enzymes like CAT, SOD and POD are formed by plants to overcome the effects of ROS. The Zn has efficient role in the formation and activity of antioxidant enzymes, and these are responsible for formation of OH^- from H_2O_2 and O_2^- [53].

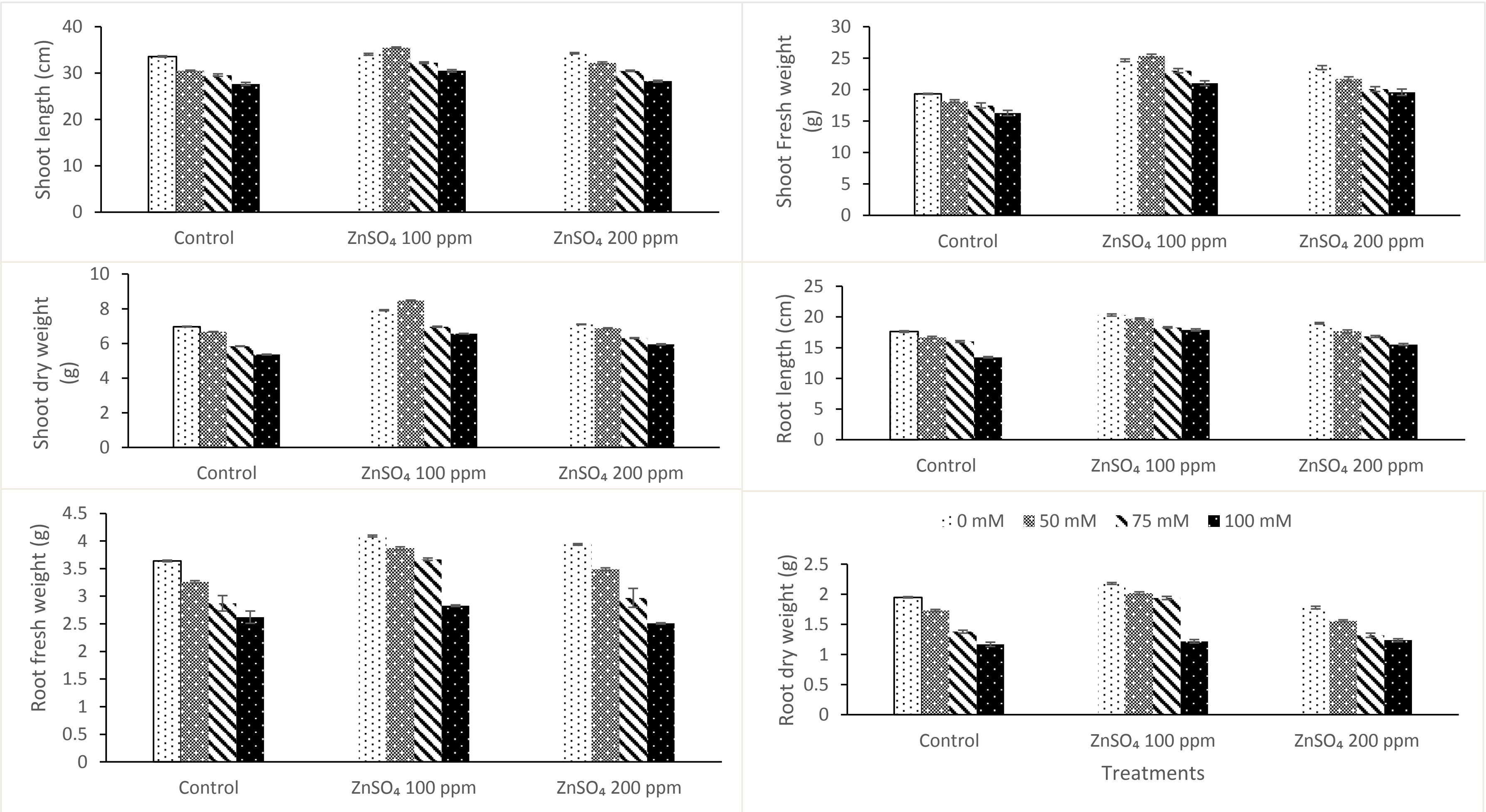

Figure 1. Effect of Nutripriming with $ZnSO_4$ on (A) Shoot length, (B) Shoot fresh weight, (C) Shoot dry weight, (D) Root length, (E) Root fresh weight & (F) Root dry weight of *Vigna radiata* L. grown under NaCl (0-50-75-100 mM) Stress

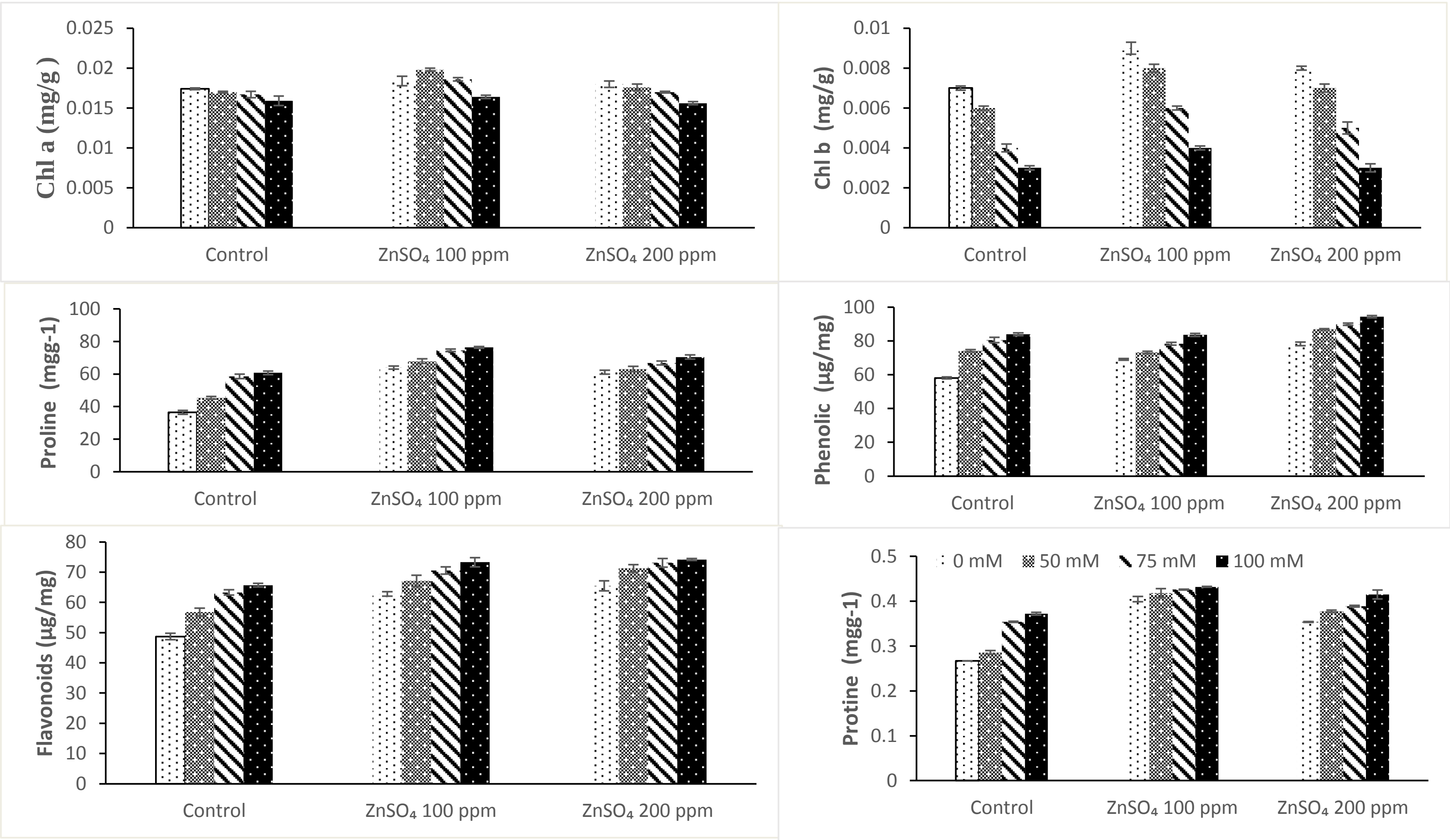

Figure 2. Effect of Nutripriming with (100ppm & 200ppm) $ZnSO_4$ on (A) Chlorophyll a, (B) Chlorophyll b, (C) Proline, (D) Phenolic contents, (E) Flavonoids & (F)Total Soluble Protein of *Vigna radiata* L. grown under NaCl (0-50-75-100 mM) Stress

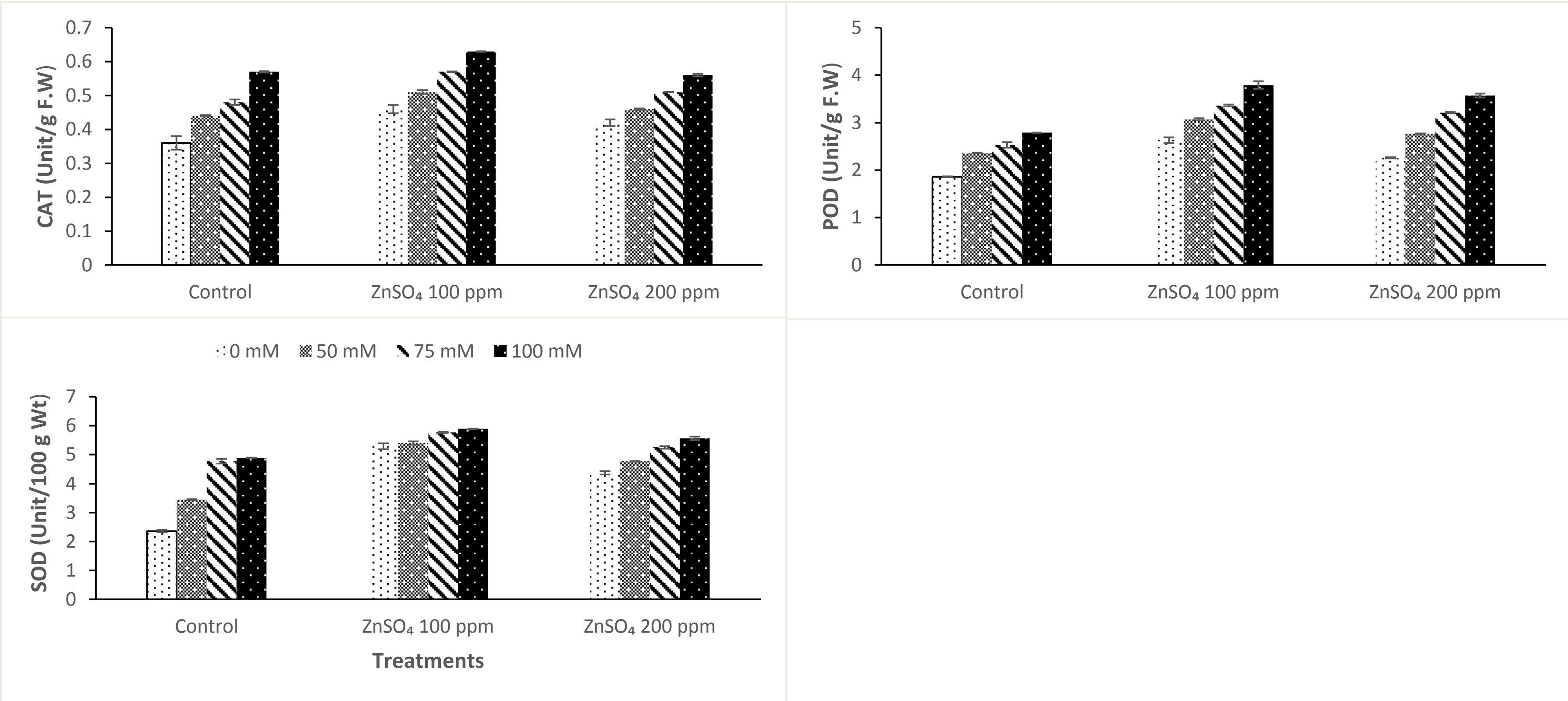

Figure 3. Effect of Nutripriming with (100ppm & 200ppm) $ZnSO_4$ on (A) CAT, (B) POD, (C) SOD activity of leaves of *Vigna radiata* L. grown under NaCl (0-50-75-100 mM) Stress

Conclusion

Overall, salt stress negatively influenced the germination and growth of *Vigna radiata* L. but antioxidant enzymes (CAT, POD, SOD) increased due to salt stress. The nutripriming with $ZnSO_4$ improved the germination percentage, growth parameters (shoot, root length, root, shoot fresh weight and dry weight) and Biochemical aspects (chlorophyll a & b, proline, protein, phenolics contents and flavonoids contents) under salt stress. Moreover, this is environment friendly technique and can be used in future.

Authors' contributions

Conceived and designed the experiment work and supervised the research work: Z Noreen, Performed the experiments and wrote article: RA Khan & TA Qadri, Analysed the data and helped in experiments: A Khan.

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